

**WHAT IS CLAIMED IS:**

1. A method of quantifying a color in a sample comprising multiple colors, the method comprising:

measuring a color channel value in a plurality of pixels from a plurality of control samples comprising a single color of interest;

defining a vector for each of the plurality of control samples, wherein each vector comprises an average of each color channel value present in the control;

defining a matrix comprising each of the averages for each of the color channels;

defining a conversion matrix comprising the inverse of the matrix based upon the control measurements;

measuring color channel values in an image of an experimental sample comprising a plurality of colors of interest, each of the pixels comprising a plurality of color channels; and

calculating the amount of a color in the experimental sample by converting the channel values in the experimental sample using the conversion matrix.

2. The method of claim 1, wherein the color channels comprise red, green, and blue.

3. The method of claim 1, wherein each control is stained with a single staining reagent to generate a color of interest.

4. The method of claim 1, wherein the experimental sample is stained with a plurality of stains to generate a plurality of colors of interest.

5. The method of claim 1, wherein the number of stains in a experimental sample are less than or equal to the number of color channels.

6. The method of claim 1, wherein an image of the experimental sample is displayed as a monochrome image.

7. The method of claim 1, further comprising setting all but one of the color channels to zero (0), thereby determining the amount of a single color in the experimental sample.

8. The method of claim 1, further comprising rendering a digital display of the experimental sample.

9. A computer implemented method of claim 1.

10. A computer program on computer readable medium comprising instructions to cause a computer to:

measure a color channel value in a plurality of pixels from a plurality of control samples comprising a single color of interest;

define a vector for each of the plurality of control samples, wherein each vector comprises an average of each color channel value present in the control;

define a matrix comprising each of the averages for each of the color channels;

define a conversion matrix comprising the inverse of the matrix based upon the control measurements;

measure color channel values in an image of an experimental sample comprising a plurality of colors of interest, each of the pixels comprising a plurality of color channels;

calculating the amount of a color in the experimental sample by converting the channel values in the experimental sample using the conversion matrix; and

outputting the amount of a color in the experimental sample.

11. The computer readable program of claim 10, wherein the color channels comprise red, green, and blue.

12. The computer readable program of claim 10, wherein each control is stained with a single staining reagent to generate a color of interest.

13. The computer readable program of claim 10, wherein the experimental sample is stained with a plurality of stains to generate a plurality of colors of interest.

14. The computer readable program of claim 10, wherein the number of stains in a experimental sample are less than or equal to the number of color channels.

15. The computer readable program of claim 10, wherein an image of the experimental sample is displayed as a monochrome image.

16. The computer readable program of claim 10, further comprising setting all but one of the color channels to zero (0), thereby determining the amount of a single color in the experimental sample.

17. The computer readable program of claim 10, further comprising rendering a digital display of the experimental sample.

18. A machine vision system for automated analysis of a biological sample on a slide comprising:

a computer comprising:

a system processor;

a computer program on computer readable medium, the computer program comprising an image algorithm comprising instructions to cause the computer to:

measure a color channel value in a plurality of pixels from a plurality of control samples comprising a single color of interest;

define a vector for each of the plurality of control samples, wherein each vector comprises an average of each color channel value present in the control;

define a matrix comprising each of the averages for each of the color channels;

define a conversion matrix comprising the inverse of the matrix based upon the control measurements;

measure color channel values in an image of an experimental sample comprising a plurality of colors of interest, each of the pixels comprising a plurality of color channels;

calculating the amount of a color in the experimental sample by converting the channel values in the experimental sample using the conversion matrix; and

outputting the amount of a color in the experimental sample;

a monitor in operable communication with the computer; and  
an input device in communication with the computer;  
an optical system in operable communication with the computer, comprising:

a movable stage;

an automated loading and unloading member for loading and unloading of a slide;

an identification member;

an optical sensing array in optical communication with the stage configured to acquire an image at a location on a slide and in electrical communication with the processor;

a storage member for storing the location of a candidate object or area of interest; and

a storage device for storing each image.

19. A method of determining cell ratio is a biological sample comprising:

calculating a ratio of positively stained cells in a sample to the total number of cells of a specified type in the sample, wherein the number of positively stained cells is determined by measuring the pixels of a first color in a digital image comprising a plurality of pixels corresponding

to the sample for a marker-specific stain divided by the total area of a field of view corresponding to the digital image, and wherein the number of total cells of a specified type is calculated by measuring the number of pixels of a reference color in a digital image comprising a plurality of pixels corresponding to the sample for cell-type stain divided by the total area of a field of view corresponding to the digital image; and

comparing the ratio to a control ratio determined with known positive to specified cell types.

20. The method of claim 19, wherein the field of view includes multiple fields of view.

21. A computer implemented method of claim 19.

22. An automated image analysis system comprising the computer implemented method of claim 9.

23. An automated image analysis system comprising the computer implemented method of claim 19.